MAURI ORA, 1981, 9: 83-95

A RE-EXAMINATION OF SOME ARID INDIAN SOILS FOR HEXOSAMINE-N CONTENT AND THE ORIGIN OF HEXOSAMINES IN MULL AND MOR SOILS

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ABSTRACT

A re-examination of the hexosamine-N content of several arid soils from Rajasthan, India has been made. Approximately 7-8% of the total nitrogen in these soils was found to occur in hexosamines. These values are considerably lower than previous estimates. The reasons for these differences are evaluated and discussed in relation to the fertility of mull and mor soil systems.

INTRODUCTION

A wide variety of plants growing on a wide variety of soils under different climatic conditions usually respond markedly to additions of fertilizer nitrogen. This is despite the fact that the soils on which these plants are growing contain many years' supply of nitrogen in the form of dead organic matter. The nature of soil nitrogen has been much studied and, although uncertainty exists concerning its origin, the bulk of the soil nitrogen is in organic form (Bremner 1967). Hydrolytic studies suggest that in surface soils approximately 5-10% of the soil nitrogen occurs in the form of hexosamines, although there has been one report indicating that 23-40% of surface soil nitrogen in arid regions is hexosamine-N (Singh and Bhandari 1962).

These high values are often quoted (e.g. Bremner 1967) and have been interpreted physico-chemically to indicate that a substantial amount of organic matter in arid regions occurs as hexosamines (Singh and Bhandari 1962). The reasons for the accumulation of hexosamines in these soils, together with the long hydrolysis period needed to release them, have been ascribed to their association with mineral, clay and humus complexes (Singh and Bhandari 1962).

During the course of researches connected with soil hexosamines, it was decided to re-examine the hexosamine-N content of arid Indian soils.

MATERIALS AND METHODS

Soil samples similar to those used by Singh and Bhandari (1962) were collected from Rajasthan by courtesy of the Indian National Bureau of Soil Survey and Land Use Planning. Samples were finely ground (53μm) and stored in air tight, dark glass jars at 6°C until required. Details of the soils are given in Table 1.

The hydrolysis of soils and hexosamine estimations were performed exactly as specified by Singh and Bhandari (1962). In addition, separate samples were hydrolysed and fractionated as specified by Bremner (1965) using a temperature of 110°C and a 24 h hydrolysis time. Due to the low nitrogen contents of many of these soils, 50 g samples and 200 ml of 6N HCl were used. After hydrolysis and filtration, hydrolysates plus washings were concentrated by rotary evaporation before neutralisation. Triplicate samples of each soil were analysed by the two methods, as were purified monomeric and polymeric hexosamines singly or admixed with the soils to determine correction factors for decomposition of hexosamines during the hydrolysis procedure (Greenfield 1979).

RESULTS

Results for hexosamine-N content are reported as a percent of the total soil N (Table 2); excellent agreement was obtained for replicates.

The hexosamine values obtained using the Bremner method are similar to those reported for soils elsewhere (Sowden *et al.*

TABLE 1. DESCRIPTION OF SOIL SAMPLES FROM RAJASTHAN

Soil number and location	Depth (cm)	Colour and texture	pH	%N
1. Kota-Nanta Farm	0-20	Dark grey clay loam to clay	7.8	0.087
2. Udaipur-Kottada Village	0-30	Reddish brown clay loam	6.9	0.042
3. Udaipur-Kawad Forest	0-22.5	Dark grey sandy loam	7.9	0.182
4. Kapasan-Kapasan Village	0-32.5	Dark grey heavy clay loam	7.2	0.070
5. Kapasan-Ballada Village	0-35	Reddish brown loam	7.3	0.101
6. Kapasan-Kapasan Tank	0-15	Yellow sandy loam	9.2	0.063
7. Jodpur- no details given	0-20	Yellow brown sandy loam	7.2	0.017

TABLE 2. SOIL HEXOSAMINE-N VALUES, AS PERCENT TOTAL SOIL NITROGEN USING THE BREMNER (1965) METHOD AND THE MODIFIED SINGH AND BHANDARI (1962) METHOD

Soil number	hexosamine-N (Bremner)	hexosamine-N (Singh and Bhandari)
1	9	8
2	10	7
3	3	3
4	7	6
5	10	9
6	4	4
7	13	11

1977), and recovery of added hexosamine-N was quantitative. In Singh and Bhandari's method, quantitative recoveries of standard hexosamines added to the soils could not be achieved and reproducibility was erratic. This was probably caused by the presence of interfering substances (such as Fe and Al), because use of longer columns (18 cm) improved the recoveries of standard hexosamines to a consistent 94%. Allowing for these recoveries, and the correction factor to compensate for hydrolytic destruction, then the hexosamine-N values were a little less than those obtained using the Bremner method. Overall, these results suggest that, with some soils, direct distillation of the hexosamine-N as NH_4-N (Bremner method) may be preferable to indirect colorimetric methods, such as that of Singh and Bhandari, which are more prone to interference.

DISCUSSION

The hexosamine-N values obtained in this study of arid Indian soils are considerably lower than those reported by Singh and Bhandari (1962). Although their results are often quoted because they are remarkably high, inspection of the original paper reveals some omissions and errors. Firstly these authors do not appear to have performed duplicate analysis on each soil and secondly they do not indicate the degree of fineness of their samples. Further, recovery rates of standard hexosamines subjected to the entire hydrolysis and fractionation procedure are not given, and the reported hexosamine values appear to have been uncorrected for losses during the hydrolysis stage. In Singh and Bhandari (1962) Table 2 column 4, the percent hexosamine values are the percent hexosamines by oven-dry weight. Column 5 of that table gives hexosamine weights and NOT hexosamine-N, as a % of total soil nitrogen. In fact, from an earlier paper (Singh and Singh 1960) on hexosamines in Uttar Pradesh soils it can be calculated (on the basis of hexosamine weight as a % of total soil nitrogen) that 32-75% of the total nitrogen in these soils occurs as "hexosamine-N". These latter values together with those reported earlier would, if correct, raise fundamental problems concerning the origin of soil nitrogen.

However, using the % N content of hexosamines and the hexosamine weights given in both papers, it is possible to calculate the proportion of total soil nitrogen that was hexosamine-N. For the surface soils used by Singh and Singh (1960) and Singh and Bhandari (1962) 3-6% and 2-3% respectively of the total nitrogen in these soils would then be hexosamine-N. Correction of these values for hydrolytic losses shows that 4-6%

of the total nitrogen in these soils, is in the form of hexosamine-N. Although these are probably underestimates, because recovery of standard hexosamines using short columns (12 cm) is not quantitative, they are similar to the values found in the present work (Table 2).

GENERAL DISCUSSION

Since 5-10% of the soil nitrogen exists in the form of hexosamines even in the contrasting soil systems of mull and mor, the origin, nature and levels of soil hexosamines seem worthy of detailed consideration. Nitrogen in hexosamines is readily transformed to mineral forms by soil micro-organisms (Bremner and Shaw 1954) and chitinases depolymerise polymeric hexosamines readily (Skujins *et al* 1965, Bloomfield and Alexander 1967). It is important to realise however, that these studies refer to hexosamines which are largely pure and that purified hexosamines are unlikely to occur in Nature (Greenfield 1979).

Living and freshly senescent plant tissues contain little hexosamine (Racusen and Foote 1974), but the cell walls of all soil micro-organisms with the exception of some lower fungi contain hexosamines complexed with various moieties (Bartnicki-Garcia 1968). Also, soil animals, particularly arthropods, possess cuticles containing hexosamines complexed to other moieties (Brown 1975). The hexosamines after extraction and purification from animal and microbial tissues, are easily biochemically decomposed as judged by the production of mineral nitrogen or monomeric hexosamines. On the other hand, and with few exceptions, when entire animal or microbial tissues collected *in vitro* or *in vivo*, or the cell walls and cuticles prepared therefrom, are subjected to chitinases, very little hexosamine-N is released in a form available for plant uptake. This seems to be due to the fact that most cuticles and cell walls are tanned and a universal property of such complexes is their great resistance to digestion by enzymes. This indicates that hexosamines occur in soils by being complexed with other substances in cell walls and cuticles. These would be present both in biomass and necromass.

The substances which complex hexosamines, e.g. carbohydrate and aromatic moieties, must be removed before hexosamines become susceptible to enzyme action. This probably occurs in two ways a) physical comminution whereby cell walls and cuticles are reduced to very small particles so that spatial barriers are removed resulting in the exposure to the enzyme

environment of a hitherto greater proportion of uncomplexed hexosamine moieties, and b) enzyme action. Allowing for temporal uncertainties this would also suggest that the correct sequence of enzymes needs to be brought into play. There is evidence to support both these viewpoints. Muller (1878, 1884) Veldkamp (1955) and Babel (1975) have commented on the presence of large amounts of pigmented microbial and insect tissues in varying degrees of comminution in soils. Most of this material appeared to consist of the remains of dead microbes and animals. However, the contribution to soil hexosamine-N from living tissues, including mycorrhizae, should not be overlooked. When more precise estimates of algae,protozoa and other soil organismsare available, it is likely that the biomass in the surface 25 cm will be several tonnes (dry weight) per hectare, even in a grassland or tundra ecosystem.

Studies (Greenfield 1981) involving the use of various enzyme sequences *in vitro* show that the various forms of nitrogen in substances such as leaf litter, insect cuticles microbial cell walls and leather can be solubilised. This process is assisted if the materials are finely divided, which is apparently an extremely important effect of the activity of soil animals on diverse organic substrates *in vivo*. The physiological condition of animal guts determines which of the animal or microbial enzymes can act on the ingested materials. Animal excrements are also constantly subjected to the activities of enzymes; or the physiological states of both systems, affected to varying degrees by the prevailing soil environmental conditions, would determine the rate, type and extent of enzyme action before further ingestion by other animals. This type of cycling and enzyme processing probably continues until sufficient exposure to a variety of enzymes removes protecting substances and allows specific enzymes to attack hexosamines. The earlier statement that the hexosamine-N values for a wide variety of soils were similar, should be interpreted cautiously. Table 3 lists the weights of total soil-N and total soil hexosamine-N for nine Rothamsted soils (1-9).* A, gives the average of these values determined on forty seven soils collected from Europe, North America, India, Africa, Australasia and Polynesia. B, records averages determined on five English and four New Zealand soils. These soils represented the mull and mor condition developed under cold and hot climates and further details are available on request to the author. For all the soils, great differences exist between the weights of

* These soils were the same as used in Table 1 of Jenkinson and Powlson's 1976 paper where a full description of these soils and their sampling is given.

TABLE 3. WEIGHTS (KG ha^{-1} 0-25 CM DEPTH) OF TOTAL SOIL N, TOTAL SOIL HEXOSAMINE-N AND BIOMASS HEXOSAMINE-N FOR ROTHAMSTED AND OTHER SOILS

Soil Number	total-N	total hexosamine-N	biomass hexosamine-N	biomass-N	Biomass-N % total N
1	2730	238	11	53	2.0
2	2440	171	9	44	2.0
3	5860	492	25	126	2.0
4	4950	416	17	84	2.0
5	3070	215	12	59	2.0
6	2620	183	10	52	2.0
7	5620	141	39	196	3.0
8	5100	428	38	190	4.0
9	5730	642	40	202	4.0
A	4520	387	51	127	3.0
range	(1620-8600)	(154-688)	(15-120)	(75-600)	
B	3450	345	83	377	11.0
range	(3100-3800)	(310-380)	(79-86)	(276-378)	

total hexosamine-N but when expressed as percentages of total soil-N they are similar i.e. 5-10%.

These latter values represent a function of the overall rate and type of metabolic activity occurring in mull and mor and characterize the different biological entities in each system. This can be demonstrated by calculating the amount of total-N in the biomass in a soil and expressing this as a percentage of the total-N in that soil. Biomass-N in soils 1-9 and A were derived from free living micro-organisms as determined by direct counting and fumigation techniques (Jenkinson and Powlson 1976, Jenkinson *et al.* 1976.) Biomass-N in B was determined from the total biomass (animals, mycorrhizae and free living micro-organisms) as determined by extraction (Phillipson 1971), dissection and scanning electron microscopy (Harley and McCready 1952, Greenfield unpublished) direct counting, fumigation and antibiotic techniques (Jenkinson and Powlson 1976, Anderson and Domsch 1975, 1978). Studies (unpublished but available for inspection on request) have shown that on average 4, 5 and 10% of the total dry weight of a wide variety of *in vivo* and *in vitro* collected mycorrhiza, free living microbes and animals respectively is nitrogen and that 30, 20 and 10% of the nitrogen in each group is in the form of hexosamine-N. For all the soils reported in Table 3, 2-4% of the total-N is in the free-living microbial biomass. However if the total-N contained in the mycorrhiza and animal biomass is included (B), then approximately 11% of the total soil-N occurs in biomass. The value of 2-4% appears to be consistent for most soils so far examined (Jenkinson and Powlson 1976, Ayanaba *et al.* 1976, Anderson and Domsch 1978, Peterson and Frederick 1979, Anderson and Domsch 1980, Lynch and Panting 1980, Clarholm and Roswall 1980, Ross *et al.* 1980). For soils where it has been possible to calculate total biomass-N, this consistency also appears, regardless of whether the soil is mull or mor. It should be stressed that estimates of microbial or animal biomass may still be prone to error, because it is not easy to discern, for example by staining methods, living tissues in soil.

If the biomass hexosamine-N values are subtracted from total soil hexosamine-N values, then a considerable amount of the soil hexosamine-N remains to be accounted. Presumably this is contained in dead animal and microbial tissues. Diamino pimelic acid (Steubing, 1970) and muramic acid (Millar and Casida 1970) are unique to bacteria and actinomycetes, and chemical estimates of these substances in soil indicate that considerably more of these substances exist than can be accounted for in biomass. Fungi and insects do not appear to contain such unique substances in their cell walls and cuticles, which can be used to differentiate them, but they do contain

chitin. By analogy with bacteria, however, considerable amounts of chemically determined hexosamine cannot be ascribed to fungal and insect biomass. Paradoxically, dead fungal and insect tissues can be isolated from soil, but until recently, direct proof for the existence of chitin in these tissues and in soils was lacking. Recent studies (Greenfield in preparation), using an alkali hydrolysis technique, have shown the existence of chitin in dead fungal and insect tissues and in soils, supporting the view that the bulk of the unaccounted hexosamine-N resides in necromass visibly identifiable or comminuted. Further support for this arises from a consideration of the total-N and hexosamine-N present in microbial cell walls and animal cuticles devoid of cytoplasmic proteins. Nitrogen accounts for approximately 3.6% of the dry weight of these tissues, and approximately 65% of this nitrogen occurs in the form of hexosamines (Greenfield unpublished but available for inspection on request). In Table 3, B soils, if the average biomass hexosamine-N is subtracted from the average total hexosamine-N then approximately 262 kg hexosamine-N remain to be accounted. Approximately 11 tonnes of dead cell walls and cuticles could account for this hexosamine-N and given that there may be 10-15 tonnes of living organisms on a hectare basis then 11 tonnes does not seem an unreasonable figure. There seems little need to invoke the humic acid concept to explain the persistence of hexosamines in soils because the chemical nature of cell walls and cuticles suggests that chitinases alone are unable to digest these materials.

In Nature, organic matter such as dead litter and dead microbial and insect tissues does not accumulate indefinitely. Enzyme systems capable of decomposing these dead tissues must occur in soil. There do not appear to be any qualitative differences between the micro-organisms in mull and mor soils on the basis of present techniques, although quantitatively there may be differences. However the types and biomass of the fauna in mulls and mors show characteristic differences. Field observations suggest that only a small proportion of the yearly litterfall is consumed by the animals in mor and that the bulk of the litter, together with animal faeces, is subjected, probably sporadically, to a microbial type of decomposition. On the other hand, the nature of the animals in mull ensures that not only is the yearly litterfall consumed at least once but that the faeces are consumed many times. Overall, dead tissues are constantly being ingested, comminuted and recycled through animal guts in mull soils, whereas such processes are unlikely to occur to anywhere near this extent in mor soils.

Animal gut possess many enzymes which act on ingested materials in a variety of ways, at the same time,depending on

the physiological conditions (e.g. redox, pH, CO_2 concentration) present in varying regions of gut. In mor soils, such persistent optimal conditions would be unlikely to occur to anywhere near the extent that they do in mulls. Microbial enzymes in mors have little opportunity to act under what may be termed physiologically acceptable conditions. It is likely that gut conditions of animals in mor and mull are qualitatively alike but differ on a quantitative basis. This suggests that dead tissues are constantly being recycled and subjected to gut enzymes in mulls. Due to the nature of the animals present in mor systems, it would be unusual for even a small proportion of the dead tissues to be recycled at the relatively high rate of recycling in mull. Although the fauna of the mor system seems to be enzymatically similar to the mull fauna both the enzyme concentrations and the frequency of enzyme contact with substrates qualitatively similar to those occurring in mull may be considerably lower. These effects will result both in different rates and types of decomposition in each soil system. The fact that similar proportions of hexosamines occur in the biomass and necromass tissues in mor and mull systems, together with the finding that the hexosamine-N as a percentage of the total-N is similar in each system, would be in accordance with the above views.

Consequently differences in the amounts of hexosamines between soils can be ascribed to quantitative differences in the cumulative hexosamine contents of the biomass and necromass present in each soil. There is no evidence to suggest that any particular group of organisms in either mull or mor possesses abnormally high levels of hexosamine.

No doubt more facts will be added to the incomplete jigsaw of soil nitrogen, but it is important to re-emphasise that care be taken to add fact rather than what appears on close inspection to be fiction. Similar views have been expressed by Romell (1935), Waksman (1938), Handley (1954) and recently by Harley (1971).

It remains that the hexosamine nitrogen of soils represents a potentially valuable source of nitrogen for plants, particularly in these times of energy problems and the insatiable demand for food and fibre. Further studies are needed to understand completely the reversibility of the mull-mor systems and the capacity of mull to supply more available nitrogen than mor. The historical evidence from forestry and agriculture indicates that mull systems can remain productive for many years without the need for regular additions of expensive fertilizer.

ACKNOWLEDGEMENT

The author thanks the Rothamsted Field Plots Committee for permission to use soil samples 1-9 (Table 3).

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Note. There appear to be several small typographical errors in the paper of Anderson and Domsch 1980. For example a forest soil has been referred to as an agricultural soil and this will affect the amounts of C and N given for that soil. The C content of the mineral soil, %C = 0.08 (Table 2) would be 1200 kg ha^{-1}. The total N content of the soils with a %N of 2.55 and 1.78 (Table 2) would appear to be 1200 and 800 kg ha^{-1} respectively. On page 215 the C contents of the soils used by Jenkinson and Powlson (1976) ranged from 0.84 to 3.49 and 1.7 to 3.7% of the total C was in the biomass. In the paper of Ayanaba et al. (1976), C content ranges from 0.72 to 1.65% and the C in the biomass was from 0.7 to 3.6% of the total. The original literature quoted by Anderson and Domsch can be checked to rectify these errors. It is stressed that these errors do not unduly affect the theme of their paper.